Identification and characterization of a novel coenzyme Q_{10}-producing strain, 
*Proteus penneri* CA8, newly isolated from Ebolowa, Cameroon

Li Zhong\(^1\), Zhuoyi Kong\(^1\), Tengfei Bian\(^2\), Changhe Cheng\(^2\), Min Shu\(^2\), Yuping Shi\(^3\), Jiale Pan\(^1\), Bidja Abena Marie Therese\(^4\) and Weihong Zhong\(^1\)*

\(^1\)College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou, China \\
\(^2\)China Tobacco Zhejiang Industrial Co. Ltd., Hangzhou, PR China \\
\(^3\)Second Affiliated Hospital Zhejiang University College of Medicine, Hangzhou, PR China \\
\(^4\)International College, Zhejiang University of Technology, Hangzhou, China

**ABSTRACT**

Coenzyme Q\(_{10}\) (CoQ\(_{10}\); also known as ubiquinone-10) is used as an oral prophylaxis and therapy for cardiovascular and mitochondrial respiratory-chain diseases, among others. This study aims to isolate and optimize the culture conditions of novel bacteria with higher coenzyme Q\(_{10}\)-production activity. A novel CoQ\(_{10}\)-producing bacterial strain, CA8, was isolated from a soil sample from Ebolowa, Cameroon using a selective medium that contained isoprene as the sole carbon source. Morphological, biochemical/physiological, and phylogenetic analyses of the 16s rRNA sequences showed that CA8 belonged to genus *Proteus*, family Enterobacteriaceae. CA8 had the closest relationship with *Proteus penneri* among all reported strains. Thus, it was identified as *P. penneri* CA8. Under optimized culture conditions, the CoQ\(_{10}\) yield of *P. penneri* CA8 reached 108.2 mg l\(^{-1}\) after 36 h of cultivation in a flask. *P. penneri* CA8 exhibited potential application in the industrial-scale biotechnological production of CoQ\(_{10}\). This report is the first one on CoQ\(_{10}\) production by *Proteus*. The potential industrial applications of CoQ\(_{10}\) production by CA8 warrant further exploration.

**Keywords:** Coenzyme *Q*\(_{10}\), Isolation, 16S rRNA, *Proteus penneri* CA8, Culture conditions

**INTRODUCTION**

Coenzyme Q\(_{10}\) (CoQ\(_{10}\); also known as ubiquinone-10) is an electron carrier with antioxidant activity and is involved in the respiratory chain. It has been successfully used as an oral prophylaxis and therapy for cardiovascular disease and mitochondrial respiratory-chain diseases, among others\(^1\). CoQ\(_{10}\) is also usually recommended as a supplement to 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), which are drugs commonly given to patients with severe heart failure \(^1,2,3,4\). The use of CoQ\(_{10}\) as a complementary therapy in heart failure increases in proportion to the growth of the aging population and the increase in statin consumption.

Production CoQ\(_{10}\) follows one of the three routes: chemical synthesis\(^5\), semichemical synthesis\(^6\), or biological synthesis\(^7\). Biological synthesis is the most viable method for industrial CoQ\(_{10}\) production. So far, a number of microorganisms have been reported as capable of producing CoQ\(_{10}\), such as *Pseudomonas* sp., *Glucosobacter* sp., *Agrobacterium* sp., *Rhodobacter* sp., *Paracoccus* sp. and *Sphingomonas* sp.\(^8,9,10,11\). The optimization of strategies and environmental parameters has resulted in improved CoQ\(_{10}\) yield in mutant strains\(^12,13,14,15\) is been chosen for further strain development. Recombinant cells of various microorganisms that contain the key genes involved in CoQ\(_{10}\) biosynthesis\(^16,17,18,19,20,21\) and in the metabolic modification of microorganisms also improve the CoQ\(_{10}\) yield\(^22,23,24,25,26\).
However, a low-cost, high-yield CoQ₁₀ production method that uses biological processes remains a major research aim in biochemical engineering [27]. Process simplification and integration are favorable pathways toward the economic production of valuable metabolites by microbes. For example, we previously designed a coupled fermentation-extraction process and succeeded to improve the CoQ₁₀ yield of *Sphingomonas* sp. ZUTE03 [11]. However, other metabolites or residual components in media can be extracted by hexane from the mixed fermentation broth phase and result in increased CoQ₁₀ purification cost. Biotransformation has several advantages over chemical synthesis including reaction specificity, regio-specificity, stereo-specificity, and mild reaction conditions [28].

Therefore, we further designed a conversion-extraction coupled process for CoQ₁₀ production by *Sphingomonas* sp. ZUTE03 directly from precursors in a two-phase conversion system. In this method, fewer metabolites are extracted into the organic phase, which leads reduced downstream purification cost [29,30]. The conversion-extraction coupled process can enhance CoQ₁₀ production in a three-phase fluidised bed reactor (TPFBR) [31]. However, the CoQ₁₀ yield by *Sphingomonas* sp. ZUTE03 is moderate compared with that in the fed-batch process in previous reports (Table 1). Further optimization, particularly in the use of microorganisms with high CoQ₁₀ content, is necessary to improve the CoQ₁₀ yield. The selection of a highly productive strain leads to higher CoQ₁₀ production at lower costs, which lead to improved industrial production by microbes.

In this study, a novel CoQ₁₀-producing strain, *Proteus penneri* CA8, with high CoQ₁₀-production activity was isolated on a selective medium from a soil sample collected from Ebolowa, Cameroon. To our knowledge, this report is the first one on CoQ₁₀ production by microbes that belong to the genus *Proteus*.

**EXPERIMENTAL SECTION**

**Chemicals**

CoQ₁₀ (>99.9 % purity) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Genomic DNA extraction and PCR kits were purchased from Invitrogen Corp. (Shanghai, China). The 16S rRNA primers were also designed and synthesized by Invitrogen Corp. All other chemicals were locally purchased.

**Media and culture conditions**

CoQ is composed of a benzoquinone ring that is prenylated with an isoprenoid chain of various lengths. CoQ₁₀-producing strains might utilize isoprene as a sole carbon source. In our previous study, a CoQ₁₀-producing strain *Sphingomonas* sp. ZUTE03 was isolated via this method [32]. Thus, a selective agar medium was designed and consisted of isoprene 0.5 g l⁻¹, 1.0 g l⁻¹ (NH₄)₂SO₄, 4.5 g l⁻¹ KH₂PO₄, 21.6 g l⁻¹ Na₂HPO₄·12H₂O, and 15–20 g l⁻¹ agar. The initial pH of this medium was adjusted to 7.0 using 2 M NaOH. The prepared medium was then autoclaved at 115 °C for 30 min.

The seed medium contained 5 g l⁻¹ yeast extract, 10 g l⁻¹ peptone, and 10 g l⁻¹ NaCl.

The initial production medium consisted of 20 g l⁻¹ glucose, 10 g l⁻¹ peptone, 10 g l⁻¹ yeast extract, 0.5 g l⁻¹ KH₂PO₄, 1.5 g l⁻¹ Na₂HPO₄, and 0.5 g l⁻¹ MgSO₄·H₂O.

To evaluate the effect of carbon source on CoQ₁₀ production by CA8, the glucose of the initial production medium was replaced by different carbon sources (glucose, sucrose, maltose, and lactose) at a concentration of 20 g l⁻¹. For evaluating the effect of nitrogen source on CoQ₁₀ production, the peptone and yeast extract of the initial production medium was replaced different nitrogen sources [(NH₄)₂SO₄, peptone, yeast extract, and com steep liquor] at a concentration of 10 g l⁻¹. For furthere evaluating the effect of the initial concentrations of lactose and yeast extract, the concentrations of lactose (10, 20, 30, 40, and 50 g l⁻¹) and yeast extract (10, 20, 30, 40, and 50 g l⁻¹) in the initial production medium were adjusted as required. One loop of strain CA8 from a slant was inoculated into a 50 ml seed medium in a 250 ml flask, and then incubated at 30 °C for 24 h. For the flask culture, 5 ml of the seed medium was inoculated in a 500 ml flask that contained 100 ml of the production medium. The culture was then grown at 30 °C and 180 rpm for 2 d.

**Isolation and identification of novel CoQ₁₀-producing strains**

Soil samples (10 g) from a corn farm in Ebolowa, Cameroon were added to a flask that contained 100 ml of physiological saline and glass beads. After shaking for 30 min at 30 °C and 180 rpm, 0.2 ml of the mixture was spread onto the solid selective plates that contained 0.5 g l⁻¹ isoprene. The plates were then incubated at 30 °C for 48 h. The colonies were collected and repeatedly streaked onto a new plate until a pure isolate was obtained. The cell morphology was examined under a transmission electron microscope. The physiological characteristics were determined using standard methods [33].
The genomic DNA of strain CA8 was extracted using a rapid extraction kit (Guangzhou Dongsheng Biotechnology Co. Ltd., China). The 16S rRNA sequence was amplified by polymerase chain reaction (PCR) using Taq polymerase (Takara, Japan) and universal primer sequences (forward primer: 5’-agagtttgatcctgctcag-3’; reverse primer: 5’-cggctacctgttacgacttc-3’). The PCR conditions were as follows: 5 min at 94 °C, 34 cycles of 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C, a final incubation at 72 °C for 10 min, and storage at -20 °C. The amplified DNA sequence was identified by Shanghai Invitrogen Biological Technique Co. Related sequences were obtained from the GenBank database using BLAST. The determined 16S rRNA gene sequences and the reference sequences obtained from GenBank databases were aligned using the CLUSTALX software. A phylogenetic tree was constructed using the MEGA4 software.

Analytical methods
To determine the CoQ10 content, the cells were harvested by centrifugation at 12000 × g at 4 °C for 15 min with a centrifuge (Sanyo, Japan). After washing the cell pellet twice with distilled water, CoQ10 was extracted with alcohol by ultrasonication at 500 W for 8 min.

CoQ10 measurements were performed according to previously reported methods[29]. The CoQ10 concentrations in all liquid samples were analysed by a high-performance liquid chromatography (HPLC) system (SPD-10AVP, SHIMADZU, Japan) equipped with an Agilent SB-C18 column (4.6 × 150 mm2). A mixture of methanol and hexane (83:17 by volume) was used as the mobile phase at a flow rate of 0.6 ml·min-1. The samples were detected using a UV detector at a wavelength of 275 nm. The sample volume was 20 µl.

CA8 cell growth was determined as the dry cell weight (DCW), which could be calculated according to a standard curve of the relationship between the cell optical density in the broth and the CA8 DCW. For the standard curve and each measurement, the cells were harvested by centrifugation (12000 × g, 10 min, 4 °C) from 2 ml of liquid culture, washed three times, and finally suspended in 5 ml of 50 mM potassium phosphate buffer (pH 7.0). The cell optical density of the suspension was determined at 600 nm using a spectrophotometer (752, Shanghai, China). The DCW for the standard curve was determined after the cells were dried overnight at 100 °C to a constant weight.

Effects of medium components and culture conditions on CoQ10 production by CA8
On a shaking flask level, the optimum carbon sources, nitrogen sources, medium volume, temperature, and pH were determined by single-factor experiments on the fermentation culture conditions. The effect of the carbon source on CoQ10 synthesis was investigated using different carbon sources (glucose, sucrose, maltose, and lactose) at a concentration of 20 g l-1. The effect of the nitrogen source on CoQ10 synthesis was determined using different nitrogen sources [(NH4)2SO4, peptone, yeast extract, and corn steep liquor] at a concentration of 10 g l-1. The initial pH of the aforementioned medium was adjusted to 7.0 using 2 M NaOH. The effects of various cultures on the CoQ10 production in 500 ml flasks were determined at 30 °C using medium volumes of 50, 80, 100, 130, and 150 ml. The effect of temperature was investigated at a cultivation pH of 7.0, and the effect of pH was investigated at a cultivation temperature of 30 °C.

Time course of CoQ10 production by CA8
Under the determined optimal culture conditions, the time course of CA8 cell growth and CoQ10 production was determined in a 500 ml flask. The sampling time intervals that were used to detect the biomass and CoQ10 content were 6 h (before 48 h culturing was reached) and 12 h (after 48 h culturing), respectively.

Statistical analysis
Each sample in the experiment was analyzed in triplicate. The Origin 8.0 software was used to draw the figures, including error bars.

Nucleotide sequence accession number
The resultant CA8 16S rRNA gene sequences were submitted to Genbank under the following accession number JX141365.
RESULTS

Isolation and identification of novel CoQ\textsubscript{10}-producing strains
Among all strains isolated from the soil samples from Ebolowa, Cameroon, the Gram-negative and spore-negative isolate CA8 was the only one with the ability to grow on a selective medium that contained isoprene as the sole carbon source. The colonies of this strain on the LB agar plate were translucent, sticky, and convex. Electron microscope observations showed that strain CA8 had rod-shaped cells with peritrichous flagella (Fig. 1). After standard biochemical/physiological identification and carbon source utilization analyses using a Biolog GEN III Microstation MicroStation, the characteristics of strain CA8 (Table 2) were found similar to those of \textit{P. penneri} or \textit{P. vulgaris}. Similarity analysis of the partial 16S rRNA gene sequence of strain CA8 (Genbank accession number: JX141365) revealed its 99\% similarity to \textit{P. penneri}. Phylogenetic tree analysis (Fig. 2) demonstrated that strain CA8 had the closest relationship with \textit{P. penneri} among all other reported strains. Therefore, strain CA8 was identified as \textit{P. penneri} CA8 and then preserved at the China Center for Type Culture Collection (Access No. CCTCC NO. M2012208).

It was reported that Enterobacteriaceae such as \textit{Proteus vulgaris} generally produces CoQ\textsubscript{8}[34]. As one species of \textit{Proteus}, it is essential to ensure CoQ type produced by \textit{Proteus penneri} CA8. Thus, an internal standard experiment of CoQ\textsubscript{10} was designed to prove the peak represented exactly CoQ\textsubscript{10}. The results (Figure 3) showed that \textit{Proteus penneri} CA8 could produce CoQ\textsubscript{10}, the peak of which was same to that of the standard CoQ\textsubscript{10} on HPLC.
Effects of carbon and nitrogen sources on CoQ$_{10}$ production by *P. penneri* CA8

Among the tested carbon sources (20 g l$^{-1}$ glucose, sucrose, maltose, and lactose), lactose exerted the greatest improvement effect on the CoQ$_{10}$ yield (35.8 mg l$^{-1}$) by CA8 (Fig. 4a). Although a higher CoQ$_{10}$ yield was observed when the initial lactose concentration was 40 g l$^{-1}$, the change in the initial lactose concentration exhibited no significant effect on CoQ$_{10}$ production by CA8 (Fig. 4b).
Peptone, yeast extract, (NH₄)₂SO₄, and corn steep liquor (10 g l⁻¹) were used to determine the effect of the nitrogen source type on CoQ₁₀ production by CA8. The results are shown in Fig. 5a. Peptone and yeast extract were more suitable than (NH₄)₂SO₄ and corn steep liquor as the nitrogen sources for CA8 cell growth and CoQ₁₀ production. However, yeast extract was the most suitable nitrogen source for CoQ₁₀ production because of its lower cost. The effect of the initial yeast extract concentration (10, 20, 30, 40, and 50 g l⁻¹) on cell growth and CoQ₁₀ production was also evaluated (Fig. 5b). The maximal CoQ₁₀ yield (84.0 mg l⁻¹) was achieved when the initial yeast extract concentration was increased to 40 g l⁻¹.

**Effects of cultivation conditions on CoQ₁₀ production by CA8**

The cultivation conditions, including the medium volume in the 500 ml flask, temperature, and initial pH, are the basic factors that affect cell growth and metabolism. Thus, the effects of these cultivation conditions were also evaluated in the flasks.

First, the effects of temperature (25, 30, and 37°C) on cell growth and CoQ₁₀ production were determined. Cell growth and CoQ₁₀ production at 30 and 37 °C were higher than that under 25°C. However, the CoQ₁₀ concentration and specific content were maximal (73.2 mg l⁻¹ and 11.1 mg g⁻¹ DCW, respectively) at 30 °C (Fig. 6a).
The effects of the medium volume (50, 80, 100, 130, and 150 ml) on CoQ<sub>10</sub> production were determined at 30 °C. The results (Fig. 6b) suggested that an extremely low or extremely high medium volume in the flask was not suitable for cell growth and CoQ<sub>10</sub> production. The maximum CoQ<sub>10</sub> concentration (88.8 g l<sup>-1</sup>) was achieved at 80 ml medium volume.

The effect of the initial medium pH (ranging from 5.0-9.0) on cell growth and CoQ<sub>10</sub> production was also determined. The results (Fig.6c) suggested that the pH range of 6–9 exerted no significant effect on cell growth. However, the maximum CoQ<sub>10</sub> concentration (105.1 mg l<sup>-1</sup>) was achieved only at pH 8.0.

In summary, the optimal cultivation conditions for CoQ<sub>10</sub> production by CA8 is 80 ml medium volume in a 500 ml flask at an initial pH of 8.0 and a cultivation temperature of 30 °C.

**Time course of CoQ<sub>10</sub> production by CA8 during flask-shaking fermentation**

The time course of cell growth and CoQ<sub>10</sub> production of CA8 under optimal culture conditions was determined. The results are shown in Fig. 7. The cell growth in the given medium showed the typical growth trend of a single-cell microorganism. However, cell growth directly entered the exponential growth phase after 24 h without a lag phase. After the cells entered the stationary phase, the CoQ<sub>10</sub> yield reached the maximal value of 108.2 mg l<sup>-1</sup> at 36 h. However, the specific yield of CoQ<sub>10</sub> reached the highest value after 12 h, suggesting that CoQ<sub>10</sub> was rapidly produced during rapid cell growth and when the total biomass remained low during the mid-exponential growth phase.

![Fig. 7 Time course of CoQ<sub>10</sub> production by CA8 during flask-shaking fermentation at 30 °C and 180 rpm. DCW (▲), CoQ<sub>10</sub> concentration (■), specific CoQ<sub>10</sub> content (●), and pH (◆). The bars represent the standard deviations of triplicate experiments](image-url)

**DISCUSSION**

Analyses of the phenotypic, biochemical/physiological, and phylogenetic characteristics of CA8 based on the 16S rRNA sequence show that the strain is a novel CoQ<sub>10</sub>-producing bacteria that should be assigned to the genus *Proteus* [35,36], family Enterobacteriaceae. The major characteristics of strain CA8 (Table 2) were similar to those of *P. penneri/vulgaris*. Similarity analysis of the partial 16S rRNA gene sequence of strain CA8 and phylogenetic tree analysis (Fig. 2) show that strain CA8 has the closest relationship with *P. penneri* among all reported strains. Therefore, strain CA8 is identified as *P. penneri CA8*.

Under the optimal culture conditions, the CoQ<sub>10</sub> yield of *P. penneri CA8* reaches 108.2 mg l<sup>-1</sup> after 36 h of fermentation in a flask. The CA8 CoQ<sub>10</sub> yield is higher than those obtained from other wild microorganisms (*Sphingomonas* sp. ZUTE03 and *Paracoccus dinitrificans* NRRL B-3785) via batch processing in a flask (Table 1). However, the yield is lower than those of other mutant microorganisms via batch processing [*Rhodobacter sphaeroides* Shenzhou6 (mutant)] and of some other wild microorganisms via the fed-batch process [*Rhodopseudomonas spheroides* (mutant)]. These results suggest that strain CA8 can improve the CoQ<sub>10</sub> yield. However, further optimization of several factors is required to improve the CoQ<sub>10</sub> yield of CA8.

First, the medium component and culture conditions of CA8 require further optimization. Second, the CoQ<sub>10</sub> production activity of the wild strain can be improved by various mutation methods, including spaceflight mutation. Third, a fed-batch process can be used to obtain a high-cell density culture of CA8, which results in a high CoQ<sub>10</sub> yield. Conversion in a two-phase system (CITPS; also called coupled conversion-extraction), which has been successfully used to improve the CoQ<sub>10</sub> yield in our previous study [11,29,31], should also be evaluated in terms of improving the CoQ<sub>10</sub> production by CA8. The addition of precursors may also improve CoQ<sub>10</sub> production by CA8 [30]. Finally, the feasibility of a scale-up process in a bioreactor coupled with a conversion-extraction process in the industrial application and economic production of CoQ<sub>10</sub> by CA8 should be investigated.
Table 1 Coenzyme Q₁₀ (CoQ₁₀) production by various microorganisms via different processes

<table>
<thead>
<tr>
<th>Process</th>
<th>Microorganism</th>
<th>CoQ₁₀ yield (mg l⁻¹)</th>
<th>Specific CoQ₁₀ yield (mg g⁻¹ DCW)</th>
<th>Working volume (l), time (h)</th>
<th>Volumetric Productivity (mg l⁻¹ h⁻¹)</th>
<th>Reference</th>
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<tr>
<td>Batch</td>
<td>Agrobacterium tumefaciens KCCM 10413</td>
<td>320</td>
<td>6.61</td>
<td>2, 96</td>
<td>3.33</td>
<td>(Ha et al. 2007)</td>
</tr>
<tr>
<td>Batch</td>
<td>Paracoccus dinitridificans NRRL B-3785</td>
<td>14.12</td>
<td>0.806</td>
<td>0.05, 109</td>
<td>0.147</td>
<td>(Bule and Singhal 2011)</td>
</tr>
<tr>
<td>Batch</td>
<td>Rhodobacter sphaeroides Shenzhou6 (mutant)</td>
<td>800</td>
<td>NG</td>
<td>NG, 168</td>
<td>4.76</td>
<td>(Lei et al. 2012)</td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Rhodopseudomonas spheroides</td>
<td>770</td>
<td>14.5</td>
<td>30, 150</td>
<td>5.13</td>
<td>(Sakato et al. 1992)</td>
</tr>
<tr>
<td>Fed-batch</td>
<td>Rhodobacter sphaeroides</td>
<td>45.65</td>
<td>4.4</td>
<td>4, 24</td>
<td>1.9</td>
<td>(Yen and Shih 2009)</td>
</tr>
<tr>
<td>Batch</td>
<td>Sphingomonas sp. ZUTE03</td>
<td>1.14</td>
<td>0.48</td>
<td>0.15, 30</td>
<td>0.04</td>
<td>(Zhong et al. 2009)</td>
</tr>
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<td>CFEPP</td>
<td>Sphingomonas sp. ZUTE03</td>
<td>43.2</td>
<td>32.5</td>
<td>0.15, 30</td>
<td>1.44</td>
<td>(Zhong et al. 2009)</td>
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<td>CTPS (free cell)</td>
<td>Sphingomonas sp. ZUTE03</td>
<td>60.8</td>
<td>40.6</td>
<td>0.15, 8</td>
<td>7.6</td>
<td>(Zhong et al. 2011)</td>
</tr>
<tr>
<td>CTPS (trap cell)</td>
<td>Sphingomonas sp. ZUTE03</td>
<td>51.6</td>
<td>38.5</td>
<td>0.15, 8</td>
<td>6.45</td>
<td>(Zhong et al. 2011)</td>
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<tr>
<td>CTPS (trap cell, repeat batch)</td>
<td>Sphingomonas sp. ZUTE03</td>
<td>&gt;40</td>
<td>&gt;23</td>
<td>0.15, 8</td>
<td>&gt;5.00</td>
<td>(Zhong et al. 2011)</td>
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<tr>
<td>CTPS (trap cell in TFPBR fed-batch)</td>
<td>Sphingomonas sp. ZUTE03</td>
<td>441.65</td>
<td>81.67</td>
<td>0.20, 144</td>
<td>3.07</td>
<td>(Qiu et al. 2012)</td>
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<tr>
<td>Batch</td>
<td>Proteus penneri CA8</td>
<td>108.2</td>
<td>11.5</td>
<td>0.08, 36</td>
<td>3.01</td>
<td>This study</td>
</tr>
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</table>

a. DCW: dry cell weight; b. CTPS: conversion in a two-phase system; c. TPFBR: three-phase fluidized bed reactor

Table 2 Physiological and biochemical characteristics of the CA8 bacterial strain

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CA8</th>
<th>Proteus vulgaris</th>
<th>Proteus mirabilis</th>
<th>Proteus penneri</th>
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<tr>
<td>Oxidase test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Voges-Proskauer test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Gelatin liquefaction (22 °C)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Indole test</td>
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<td>+</td>
<td>-</td>
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<td>Phenylalanine deaminase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Citrate utilisation (Simmons)</td>
<td>-</td>
<td>v</td>
<td>+</td>
<td>v</td>
</tr>
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<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>v</td>
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<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Salicylic acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Cellulose</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Glycerol</td>
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<td>D-Mannitol</td>
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<tr>
<td>D-Mannose</td>
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<td>L-Rhamnose</td>
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<td>D-Sorbitol</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>v</td>
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</table>

+, Positive; -, negative; v, variable between strains

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